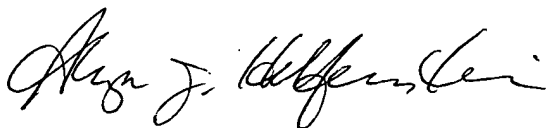


## REMARKS

The above preliminary amendment is proposed to eliminate the redundant citation of two patent references, one of which is also incorrectly cited. If there are any questions regarding this paper or the application in general, the Examiner is urged to contact the undersigned attorney so that allowance of the application may be facilitated.

Respectfully submitted,



Allegra J. Helfenstein.  
Registration No. 34,179  
Legal Counsel  
Molecular Probes, Inc.  
P.O. Box 22010  
Eugene, OR 97402-0469  
TEL: (541) 465-8362  
FAX: (541) 465-8354



VERSION WITH MARKINGS TO SHOW CHANGES MADE

(changes are indicated by brackets and strikethrough)

A variety of fluorescent indicators that are useful for the detection of biologically relevant soluble free metal ions (such as  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$ ) have been described that utilize oxygen-containing anionic or polyanionic chelators to bind to metal ions. In particular, fluorescent indicators utilizing a polycarboxylate BAPTA chelator have been previously described (U.S. Patent No.: 4,603,209 to Tsien et al. (1986); U.S. Patent No. 5,049,673 to Tsien et al. (1991); U.S. Patent No. 4,849,362 to DeMarinis et al. (1989); U.S. Patent No. 5,453,517 to Kuhn et al. (1995); [~~U.S. Patent No. 5,501,980 to Malekzadeh et al. (1996); U.S. Patent No. 5,459,276 to Kuhn et al. (1995);~~] U.S. Patent No. 5,501,980 to Katerinopoulos et al. (1996); U.S. Patent No. 5,459,276 to Kuhn et al. (1995) (all incorporated by reference). Some fluorescent indicators selective for  $\text{Li}^+$ ,  $\text{Na}^+$  and  $\text{K}^+$  in aqueous or organic solution have also been described, based on the chemical modification of crown ethers (U.S. Patent No. 5,134,232 to Tsien et al. (1992); U.S. Patent No. 5,405,975 to Kuhn et al. (1995); both incorporated by reference).